

## PR60235USW SEQ LIST

## SEQUENCE LISTING

<110> SmithKline Beecham Corporation  
 Lambert, Millard H  
 Xu, Robert  
 Wisely, Bruce  
 Collins, Jon

<120> CAR LIGAND-BINDING DOMAIN POLYPEPTIDE CO-CRYSTALLIZED WITH A  
 LIGAND, AND METHODS OF DESIGNING LIGANDS THAT MODULATE CAR  
 ACTIVITY

<130> PR60235

<150> 60/488,415

<151> 2003-07-18

<160> 7

<170> PatentIn version 3.2

<210> 1

<211> 1450

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (273)..(1316)

<400> 1

gtgagcttgc tccttaagtt acaggaactc tccttataat agacacttca ttttcctagt 60

ccatccctca tgaaaaatga ctgaccactg ctgggcagca ggagggatga taatcctaac 120

tccaatcact ggcaactcct gagatcagag gaaaaccagc aacagcgtgg gagtttgggg 180

agaggcattc cataccagat tctgtggcct gcaggtgaca tgctgcctaa gagaagcagg 240

agtctgtgac agccacccca acacgtgacg tc atg gcc agt agg gaa gat gag 293  
 Met Ala Ser Arg Glu Asp Glu  
 1 5

ctg agg aac tgt gtg gta tgt ggg gac caa gcc aca ggc tac cac ttt 341  
 Leu Arg Asn Cys Val Val Cys Gly Asp Gln Ala Thr Gly Tyr His Phe  
 10 15 20

aat gcg ctg act tgt gag ggc tgc aag ggt ttc ttc agg aga aca gtc 389  
 Asn Ala Leu Thr Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg Thr Val  
 25 30 35

agc aaa agc att ggt ccc acc tgc ccc ttt gct gga agc tgt gaa gtc 437  
 Ser Lys Ser Ile Gly Pro Thr Cys Pro Phe Ala Gly Ser Cys Glu Val  
 40 45 50 55

agc aag act cag agg cgc cac tgc cca gcc tgc agg ttg cag aag tgc 485  
 Ser Lys Thr Gln Arg Arg His Cys Pro Ala Cys Arg Leu Gln Lys Cys  
 60 65 70

tta gat gct ggc atg agg aaa gac atg ata ctg tcg gca gaa gcc ctg 533  
 Leu Asp Ala Gly Met Arg Lys Asp Met Ile Leu Ser Ala Glu Ala Leu  
 75 80 85

## PR60235USW SEQ LIST

gca	ttg	cgg	cga	gca	aag	cag	gcc	cag	cgg	cgg	gca	cag	caa	aca	cct	581
Ala	Leu	Arg	Arg	Ala	Lys	Gln	Ala	Gln	Arg	Arg	Ala	Gln	Gln	Thr	Pro	
	90						95				100					
gtg	caa	ctg	agt	aag	gag	caa	gaa	gag	ctg	atc	cgg	aca	ctc	ctg	ggg	629
Val	Gln	Leu	Ser	Lys	Glu	Gln	Glu	Glu	Leu	Ile	Arg	Thr	Leu	Leu	Gly	
	105					110					115					
gcc	cac	acc	cgc	cac	atg	ggc	acc	atg	ttt	gaa	cag	ttt	gtg	cag	ttt	677
Ala	His	Thr	Arg	His	Met	Gly	Thr	Met	Phe	Glu	Gln	Phe	Val	Gln	Phe	
	120				125					130					135	
agg	cct	cca	gct	cat	ctg	ttc	atc	cat	cac	cag	ccc	ttg	ccc	acc	ctg	725
Arg	Pro	Pro	Ala	His	Leu	Phe	Ile	His	His	Gln	Pro	Leu	Pro	Thr	Leu	
				140					145					150		
gcc	cct	gtg	ctg	cct	ctg	gtc	aca	cac	ttc	gca	gac	atc	aac	act	ttc	773
Ala	Pro	Val	Leu	Pro	Leu	Val	Thr	His	Phe	Ala	Asp	Ile	Asn	Thr	Phe	
			155					160					165			
atg	gta	ctg	caa	gtc	atc	aag	ttt	act	aag	gac	ctg	ccc	gtc	ttc	cgt	821
Met	Val	Leu	Gln	Val	Ile	Lys	Phe	Thr	Lys	Asp	Leu	Pro	Val	Phe	Arg	
		170					175					180				
tcc	ctg	ccc	att	gaa	gac	cag	atc	tcc	ctt	ctc	aag	gga	gca	gct	gtg	869
Ser	Leu	Pro	Ile	Glu	Asp	Gln	Ile	Ser	Leu	Leu	Lys	Gly	Ala	Ala	Val	
	185					190					195					
gaa	atc	tgt	cac	atc	gta	ctc	aat	acc	act	ttc	tgt	ctc	caa	aca	caa	917
Glu	Ile	Cys	His	Ile	Val	Leu	Asn	Thr	Thr	Phe	Cys	Leu	Gln	Thr	Gln	
	200				205					210					215	
aac	ttc	ctc	tgc	ggg	cct	ctt	cgc	tac	aca	att	gaa	gat	gga	gcc	cgt	965
Asn	Phe	Leu	Cys	Gly	Pro	Leu	Arg	Tyr	Thr	Ile	Glu	Asp	Gly	Ala	Arg	
				220				225						230		
gtg	ggg	ttc	cag	gta	gag	ttt	ttg	gag	ttg	ctc	ttt	cac	ttc	cat	gga	1013
Val	Gly	Phe	Gln	Val	Glu	Phe	Leu	Glu	Leu	Leu	Phe	His	Phe	His	Gly	
			235					240					245			
aca	cta	cga	aaa	ctg	cag	ctc	caa	gag	cct	gag	tat	gtg	ctc	ttg	gct	1061
Thr	Leu	Arg	Lys	Leu	Gln	Leu	Gln	Glu	Pro	Glu	Tyr	Val	Leu	Leu	Ala	
		250					255					260				
gcc	atg	gcc	ctc	ttc	tct	cct	gac	cga	cct	gga	gtt	acc	cag	aga	gat	1109
Ala	Met	Ala	Leu	Phe	Ser	Pro	Asp	Arg	Pro	Gly	Val	Thr	Gln	Arg	Asp	
	265					270					275					
gag	att	gat	cag	ctg	caa	gag	gag	atg	gca	ctg	act	ctg	caa	agc	tac	1157
Glu	Ile	Asp	Gln	Leu	Gln	Glu	Glu	Met	Ala	Leu	Thr	Leu	Gln	Ser	Tyr	
	280				285					290					295	
atc	aag	ggc	cag	cag	cga	agg	ccc	cgg	gat	cgg	ttt	ctg	tat	gcg	aag	1205
Ile	Lys	Gly	Gln	Gln	Arg	Arg	Pro	Arg	Asp	Arg	Phe	Leu	Tyr	Ala	Lys	
				300				305						310		
ttg	cta	ggc	ctg	ctg	gct	gag	ctc	cgg	agc	att	aat	gag	gcc	tac	ggg	1253
Leu	Leu	Gly	Leu	Leu	Ala	Glu	Leu	Arg	Ser	Ile	Asn	Glu	Ala	Tyr	Gly	
			315					320					325			
tac	caa	atc	cag	cac	atc	cag	ggc	ctg	tct	gcc	atg	atg	ccg	ctg	ctc	1301
Tyr	Gln	Ile	Gln	His	Ile	Gln	Gly	Leu	Ser	Ala	Met	Met	Pro	Leu	Leu	

PR60235USW SEQ LIST

330

335

340

cag gag atc tgc agc tgaggccatg ctcaattcct tccccagctc acctggaaca 1356  
 Gln Glu Ile Cys Ser  
 345  
 ccctggatac actggagtg gaaaatgctg ggaccaaaga ttgggccggg ttcaaaggga 1416  
 gcccgatggt tgcaatgaaa gactaaagca aaac 1450

<210> 2  
 <211> 348  
 <212> PRT  
 <213> Homo sapiens  
 <400> 2

Met Ala Ser Arg Glu Asp Glu Leu Arg Asn Cys Val Val Cys Gly Asp  
 1 5 10 15  
 Gln Ala Thr Gly Tyr His Phe Asn Ala Leu Thr Cys Glu Gly Cys Lys  
 20 25 30  
 Gly Phe Phe Arg Arg Thr Val Ser Lys Ser Ile Gly Pro Thr Cys Pro  
 35 40 45  
 Phe Ala Gly Ser Cys Glu Val Ser Lys Thr Gln Arg Arg His Cys Pro  
 50 55 60  
 Ala Cys Arg Leu Gln Lys Cys Leu Asp Ala Gly Met Arg Lys Asp Met  
 65 70 75 80  
 Ile Leu Ser Ala Glu Ala Leu Ala Leu Arg Arg Ala Lys Gln Ala Gln  
 85 90 95  
 Arg Arg Ala Gln Gln Thr Pro Val Gln Leu Ser Lys Glu Gln Glu Glu  
 100 105 110  
 Leu Ile Arg Thr Leu Leu Gly Ala His Thr Arg His Met Gly Thr Met  
 115 120 125  
 Phe Glu Gln Phe Val Gln Phe Arg Pro Pro Ala His Leu Phe Ile His  
 130 135 140  
 His Gln Pro Leu Pro Thr Leu Ala Pro Val Leu Pro Leu Val Thr His  
 145 150 155 160  
 Phe Ala Asp Ile Asn Thr Phe Met Val Leu Gln Val Ile Lys Phe Thr  
 165 170 175  
 Lys Asp Leu Pro Val Phe Arg Ser Leu Pro Ile Glu Asp Gln Ile Ser  
 180 185 190  
 Leu Leu Lys Gly Ala Ala Val Glu Ile Cys His Ile Val Leu Asn Thr  
 195 200 205  
 Thr Phe Cys Leu Gln Thr Gln Asn Phe Leu Cys Gly Pro Leu Arg Tyr  
 210 215 220

PR60235USw SEQ LIST

Thr Ile Glu Asp Gly Ala Arg Val Gly Phe Gln Val Glu Phe Leu Glu  
 225 230 235 240

Leu Leu Phe His Phe His Gly Thr Leu Arg Lys Leu Gln Leu Gln Glu  
 245 250 255

Pro Glu Tyr Val Leu Leu Ala Ala Met Ala Leu Phe Ser Pro Asp Arg  
 260 265 270

Pro Gly Val Thr Gln Arg Asp Glu Ile Asp Gln Leu Gln Glu Glu Met  
 275 280 285

Ala Leu Thr Leu Gln Ser Tyr Ile Lys Gly Gln Gln Arg Arg Pro Arg  
 290 295 300

Asp Arg Phe Leu Tyr Ala Lys Leu Leu Gly Leu Leu Ala Glu Leu Arg  
 305 310 315 320

Ser Ile Asn Glu Ala Tyr Gly Tyr Gln Ile Gln His Ile Gln Gly Leu  
 325 330 335

Ser Ala Met Met Pro Leu Leu Gln Glu Ile Cys Ser  
 340 345

<210> 3  
 <211> 714  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> (1)..(714)

<400> 3  
 cct gtg caa ctg agt aag gag caa gaa gag ctg atc cgg aca ctc ctg 48  
 Pro Val Gln Leu Ser Lys Glu Gln Glu Glu Leu Ile Arg Thr Leu Leu  
 1 5 10

ggg gcc cac acc cgc cac atg ggc acc atg ttt gaa cag ttt gtg cag 96  
 Gly Ala His Thr Arg His Met Gly Thr Met Phe Glu Gln Phe Val Gln  
 20 25 30

ttt agg cct cca gct cat ctg ttc atc cat cac cag ccc ttg ccc acc 144  
 Phe Arg Pro Pro Ala His Leu Phe Ile His His Gln Pro Leu Pro Thr  
 35 40 45

ctg gcc cct gtg ctg cct ctg gtc aca cac ttc gca gac atc aac act 192  
 Leu Ala Pro Val Leu Pro Leu Val Thr His Phe Ala Asp Ile Asn Thr  
 50 55 60

ttc atg gta ctg caa gtc atc aag ttt act aag gac ctg ccc gtc ttc 240  
 Phe Met Val Leu Gln Val Ile Lys Phe Thr Lys Asp Leu Pro Val Phe  
 65 70 75 80

cgt tcc ctg ccc att gaa gac cag atc tcc ctt ctc aag gga gca gct 288  
 Arg Ser Leu Pro Ile Glu Asp Gln Ile Ser Leu Leu Lys Gly Ala Ala  
 85 90 95

gtg gaa atc tgt cac atc gta ctc aat acc act ttc tgt ctc caa aca 336  
 Val Glu Ile Cys His Ile Val Leu Asn Thr Thr Phe Cys Leu Gln Thr  
 100 105 110

PR60235USw SEQ LIST

caa	aac	ttc	ctc	tgc	ggg	cct	ctt	cgc	tac	aca	att	gaa	gat	gga	gcc	384
Gln	Asn	Phe	Leu	Cys	Gly	Pro	Leu	Arg	Tyr	Thr	Ile	Glu	Asp	Gly	Ala	
		115					120					125				
cgt	gtg	ggg	ttc	cag	gta	gag	ttt	ttg	gag	ttg	ctc	ttt	cac	ttc	cat	432
Arg	Val	Gly	Phe	Gln	Val	Glu	Phe	Leu	Glu	Leu	Leu	Phe	His	Phe	His	
	130					135					140					
gga	aca	cta	cga	aaa	ctg	cag	ctc	caa	gag	cct	gag	tat	gtg	ctc	ttg	480
Gly	Thr	Leu	Arg	Lys	Leu	Gln	Leu	Gln	Glu	Pro	Glu	Tyr	Val	Leu	Leu	
145					150					155					160	
gct	gcc	atg	gcc	ctc	ttc	tct	cct	gac	cga	cct	gga	gtt	acc	cag	aga	528
Ala	Ala	Met	Ala	Leu	Phe	Ser	Pro	Asp	Arg	Pro	Gly	Val	Thr	Gln	Arg	
				165					170					175		
gat	gag	att	gat	cag	ctg	caa	gag	gag	atg	gca	ctg	act	ctg	caa	agc	576
Asp	Glu	Ile	Asp	Gln	Leu	Gln	Glu	Glu	Met	Ala	Leu	Thr	Leu	Gln	Ser	
			180					185					190			
tac	atc	aag	ggc	cag	cag	cga	agg	ccc	cgg	gat	cgg	ttt	ctg	tat	gcg	624
Tyr	Ile	Lys	Gly	Gln	Gln	Arg	Arg	Pro	Arg	Asp	Arg	Phe	Leu	Tyr	Ala	
		195					200					205				
aag	ttg	cta	ggc	ctg	ctg	gct	gag	ctc	cgg	agc	att	aat	gag	gcc	tac	672
Lys	Leu	Leu	Gly	Leu	Leu	Ala	Glu	Leu	Arg	Ser	Ile	Asn	Glu	Ala	Tyr	
	210					215					220					
ggg	tac	caa	atc	cag	cac	atc	cag	ggc	ctg	tct	gcc	atg	atg			714
Gly	Tyr	Gln	Ile	Gln	His	Ile	Gln	Gly	Leu	Ser	Ala	Met	Met			
225					230					235						

<210> 4  
 <211> 238  
 <212> PRT  
 <213> Homo sapiens

<400> 4

Pro	Val	Gln	Leu	Ser	Lys	Glu	Gln	Glu	Glu	Leu	Ile	Arg	Thr	Leu	Leu	
1				5					10					15		
Gly	Ala	His	Thr	Arg	His	Met	Gly	Thr	Met	Phe	Glu	Gln	Phe	Val	Gln	
			20					25					30			
Phe	Arg	Pro	Pro	Ala	His	Leu	Phe	Ile	His	His	Gln	Pro	Leu	Pro	Thr	
		35					40					45				
Leu	Ala	Pro	Val	Leu	Pro	Leu	Val	Thr	His	Phe	Ala	Asp	Ile	Asn	Thr	
	50					55					60					
Phe	Met	Val	Leu	Gln	Val	Ile	Lys	Phe	Thr	Lys	Asp	Leu	Pro	Val	Phe	
65				70						75					80	
Arg	Ser	Leu	Pro	Ile	Glu	Asp	Gln	Ile	Ser	Leu	Leu	Lys	Gly	Ala	Ala	
				85					90					95		
Val	Glu	Ile	Cys	His	Ile	Val	Leu	Asn	Thr	Thr	Phe	Cys	Leu	Gln	Thr	
			100					105					110			
Gln	Asn	Phe	Leu	Cys	Gly	Pro	Leu	Arg	Tyr	Thr	Ile	Glu	Asp	Gly	Ala	
		115					120						125			

PR60235USw SEQ LIST

Arg Val Gly Phe Gln Val Glu Phe Leu Glu Leu Leu Phe His Phe His  
 130 135 140  
 Gly Thr Leu Arg Lys Leu Gln Leu Gln Glu Pro Glu Tyr Val Leu Leu  
 145 150 155 160  
 Ala Ala Met Ala Leu Phe Ser Pro Asp Arg Pro Gly Val Thr Gln Arg  
 165 170 175  
 Asp Glu Ile Asp Gln Leu Gln Glu Glu Met Ala Leu Thr Leu Gln Ser  
 180 185 190  
 Tyr Ile Lys Gly Gln Gln Arg Arg Pro Arg Asp Arg Phe Leu Tyr Ala  
 195 200 205  
 Lys Leu Leu Gly Leu Leu Ala Glu Leu Arg Ser Ile Asn Glu Ala Tyr  
 210 215 220  
 Gly Tyr Gln Ile Gln His Ile Gln Gly Leu Ser Ala Met Met  
 225 230 235

<210> 5  
 <211> 11  
 <212> PRT  
 <213> Artificial

<220>  
 <223> Amino acid sequence of an artificial His tag  
 <400> 5

Met Lys Lys Gly His His His His His His Gly  
 1 5 10

<210> 6  
 <211> 69  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Forward primer for amplifying amino acids 103-348, including  
 sequences encoding a His tag, and including an NdeI restriction  
 site

<400> 6  
 cggcggcgcc atatgaaaaa aggtcatcat catcatcatc atggtcctgt gcaactgagt 60  
 aaggagcaa 69

<210> 7  
 <211> 45  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Reverse primer for amplifying amino acids 103-348, incorporating  
 a BamHI site

<400> 7  
 cggcggcgcg gatccttatt agctgcagat ctctgggagc agcgg 45